TAB 2

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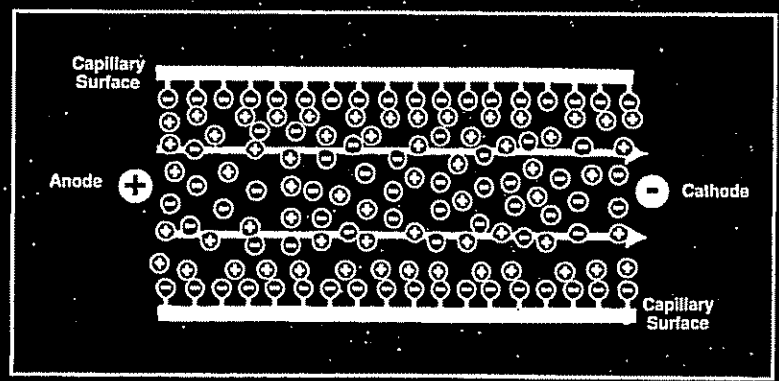
CAPILLARY ELECTROPHORESIS GUIDEBOOK

Principles, Operation, and Applications

Edited by

Kevin D. Altria



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CHAPTER 13

Capillary Gel Electrophoresis

András Guttman

1. Introduction

There is a great deal of interest in analytical biochemistry in the separation and identification of biologically important polymers, such as DNA protein and complex carbohydrate molecules *(1,2)*. For relatively short single-stranded DNA units (i.e., oligonucleotides) and carbohydrate molecules, there is a need to separate by a single base difference (for DNA sequencing) *(3)* or even for identical chain length with a different sequence (identification of primers, probes, and antisense DNA molecules) *(3,4)*. For the double-stranded DNA molecules, there is an interest to analyze and identify DNA molecules in the form of restriction fragments or PCR products. Using various types of sieving media allows us to do these kinds of separations. In capillary gel electrophoresis, crosslinked or noncrosslinked sieving matrices can be employed *(5–7)*. The crosslinked gels, i.e., chemical gels, have a well-defined pore size. Noncrosslinked, or so-called physical gels, have a dynamic pore structure. This major difference provides the noncrosslinked linear polymer networks with much higher flexibility when compared to the crosslinked gels. One can operate at high temperatures (up to 50–70°C) while applying extremely high field strengths (up to 10^3 V/cm range) without any damage to the linear polymer network formulations *(8)*. It is important to note that the crosslinked gels are not usable under such extreme conditions *(9)*. The other main advantage of the linear polymer network system is that it can be easily replaced in the capillary column by simply

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rinsing the gel matrix through the capillary by pressure or vacuum. Therefore, if the column becomes contaminated, the gel is easily replaced extending the lifetime of the system. Employing the replaceable concept, there is a possibility of the use of pressure injection compared to the crosslinked gels where electrokinetic injection mode is the only possibility *(10)*. It is important to note that in addition to convenience, pressure injection permits quantitative analysis.

2. Materials

2.1. Apparatus

In all these studies, the power supply of the capillary electrophoresis apparatus (homemade or commercial) was used in reversed polarity mode, with the cathode on the injection side and the anode on the detection side. The separations were monitored on-column at 214 nm for the protein and carbohydrate, and at 254 nm for the DNA and the dansylated amino acid samples. The temperature of the gel-filled capillary columns was maintained in all experiments at 20°C ± 0.5°C even at high field strengths by the Peltier device controlled cooling system *(11)*. The electropherograms were acquired and stored on an Everex 386/33 computer using the System Gold™ software package (Beckman Instruments, Inc., Fullerton, CA).

2.2. Chemicals

The crude 70-mer and the slab-gel-purified 99-mer oligonucleotides were the gift of N. Bischoffer (Genentech, South San Francisco, CA). The human K-*ras* oncogenes (dGTTGGAGCT-C-GTGGCGTAG, dGTTGGAGCT-G-GTGGCGTAG, dGTTGGAGCT-T-GTGGCGTAG) were purchased from Pharmacia (Piscataway, NJ). The DNA restriction fragment mixture, ϕX174 DNA-*Hae*III digest, was purchased from New England Biolabs (Beverly, MA). The 102-mer was synthetized in-house. All the DNA samples were diluted to 50 μg/mL with water before injection. Ultrapure electrophoresis grade acrylamide, Tris, boric acid, EDTA, urea, ammonium persulfate, and TEMED were employed in the experiments (Schwartz/Mann Biotech, Cambridge, MA). Orange G (Sigma, St. Louis, MO) was used in the electrophoretic separations as an internal standard at 0.01% concentration.

The dansylated D,L-amino acids (Dns-DL-AA) and the SDS protein test mixture (14,400–97,400 Dalton) were purchased from Sigma. ANTS-